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Hyaluronic Acid and Fibrin Hydrogels with Concentrated DNA/PEI Polyplexes for Local Gene Delivery

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Abstract

Local delivery of DNA through a hydrogel scaffold would increase the applicability of gene therapy in tissue regeneration and cancer therapy. However, the delivery of DNA/cationic polymer nanoparticles (polyplexes) using hydrogels is challenging due to the aggregation and inactivation of polyplexes during their incorporation into hydrogel scaffolds. We developed a novel process (termed Caged Nanoparticle Encapsulation or CnE) to load concentrated and unaggregated non-viral gene delivery nanoparticles into various hydrogels. Previously, we showed that PEG hydrogels loaded with DNA/PEI polyplexes through this process were able to deliver genes both *in vitro* and *in vivo*. In this study, we found that hyaluronic acid and fibrin hydrogels with concentrated and unaggregated polyplexes loaded through CnE were able to deliver genes *in vivo* as well, demonstrating the universality of the process.

Keywords

Gene transfer; Gene delivery; Hyaluronic acid hydrogel; Fibrin hydrogel; Scaffold-mediated gene delivery

Introduction

Gene therapy is the genetic modification of cells through the delivery of DNA or RNA (e.g. small interfering RNA, siRNA), which can up- or down-regulate genes respectively. Gene therapy can be achieved with the use of modified viruses (viral delivery) or non-viral gene delivery vectors (non-viral delivery) that encapsulate or condense plasmid DNA or siRNA into nanoparticles that transport the genetic material into the cell [1–4]. Although non-viral delivery is generally less efficient than viral delivery *in vivo*, it has the following advantages: (1) the large-scale production of non-viral gene delivery nanoparticles can be more readily accomplished than that for viruses, (2) repeated injection non-viral nanoparticles is possible since they can be engineered not to elicit a severe immune response, (3) the risk of insertion mutagenesis is low since the non-viral gene delivery vectors do not integrate the delivered DNA into the genomic DNA and (4) larger genes can be delivered since the non-viral vectors can complex with large DNA molecules.

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Local delivery of DNA or siRNA through a hydrogel scaffold would increase the applicability of gene therapy in tissue regeneration and cancer therapy [5,6]. Genes encoding for tissue inductive factors can be delivered to the diseased site with the use of a hydrogel scaffold. DNA is transferred to the infiltrating cells to produce protein factors *in situ* to promote tissue regeneration [6,7]. For cancer therapy, lethal genes or siRNAs can be delivered with a hydrogel scaffold to induce the apoptosis of tumor cells [8]. Naked DNA was first incorporated into hydrogels, such as collagen [9–11], pluronic-hyaluronic acid [12], PEG-poly(lactic acid)-PEG [13], alginate [14], oligo(polyethylene glycol) fumarate [15] and engineered silk elastin [16] for local gene therapy. Although naked DNA encapsulated in collagen hydrogels was able to promote bone regeneration *in vivo* [9,17], the gene transfer efficiency was very low and most of the encapsulated DNA diffused out from the hydrogel scaffold rapidly. This motivated researchers to use non-viral gene delivery vectors to condense DNA into nanoparticles. These nanoparticles can both retain the DNA in the scaffold and significantly enhance the gene transfer efficiency. Cationic peptides, lipids, or polymers have been used to prepare nanoparticles that were encapsulated into collagen [18–20], fibrin [21–26], enzymatically degradable PEG hydrogels [27] and PEG-hyaluronic acid [22] hydrogels. Although gene delivery has been observed from these hydrogels, limitations with low DNA loading and low transgene expression motivated us to find an effective approach for introducing DNA/cationic polymer polyplexes into hydrogel scaffolds.

Previously, we reported on a strategy to incorporate concentrated, un-aggregated and highly active DNA/PEI polyplexes inside polyethylene glycol (PEG) hydrogel scaffolds, termed Caged Nanoparticle Encapsulation or CnE [28]. The approach utilizes neutral saccharides (sucrose) and polysaccharides (agarose) to protect the polyplexes from aggregation and inactivation during lyophilization and hydrogel formation, respectively. Results showed that matrix metalloproteinase (MMP) degradable polyethylene glycol (PEG) hydrogels with concentrated DNA/PEI polyplexes loaded through CnE were able to deliver genes *in vivo* and induced extensive angiogenesis when genes encoding for VEGF were delivered. In this report, we investigated whether CnE could be used to introduce active DNA/PEI polyplexes into charged hyaluronic acid and protein fibrin hydrogels and achieve efficient gene transfer *in vivo*. Both HA and fibrin pose extreme challenges in incorporating PEI/DNA polyplexes. HA is highly negatively charged and would compete with DNA for the positively charged PEI, causing polyplex decomplexation. Fibrin is a protein that can absorb the polyplexes and cause aggregation.

Materials and methods

Materials

Human fibrinogen, Bovine plasma thrombin and Linear poly(ethylene imine) (25 kDa, PEI) were bought from Enzyme Research Laboratories, Sigma and Polysciences, respectively. Vectors expressing vascular endothelial growth factor (pVEGF) [29] and beta-galactosidase (p β gal) were gifts from Prof. Lonnie Shea's laboratory (Northwestern University). pVEGF, has the VEGF gene is inserted in a pcD3.1 vector with a CMV promoter. p β gal has the β gal gene inserted in a pNGVL1 promoter with a CMV promoter. A Giga Prep kit from Qiagen was used to expand the plasmid. Sodium hyaluronan (HA) was a kind gift from Genzyme Corporation (60 KDa MW, Cambridge, MA). All other chemicals were purchased from Fisher Scientific unless otherwise noted.

Hyaluronic acid modification

Acrylated hyaluronic acid (HA-AC) was prepared as previously described [28]. Briefly, 1.0 g hyaluronic acid was reacted with 18.0 g adipic dihydrazide (ADH) at pH 4.75 in the

presence of 2.0 g 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) overnight and purified through dialysis (8000 MWCO) in DI water for 1 week. 38.8% of the carboxyl groups were modified with ADH based on the TNBSA assay. One gram of HA-ADH was reacted with 0.75 g N-Acryloxysuccinimide in HEPES buffer (pH 7.2) overnight and purified through dialysis in DI water for 1 week. All the primary amines were acrylated based on the 2,4,6-Trinitrobenzene sulfonic acid (TNBSA, Thermo Scientific) assay performed following the product manual.

Polyplex lyophilization

Plasmid DNA (0–300 µg) and PEI (0–587 µg) were mixed in 3.5 mL water in the presence of 35 mg (0.10 mmole) of sucrose (Ultra pure, MP Biomedicals) and incubated at room temperature for 15 min. Agarose (1.0 mg, UltraPure™ Agarose, Tm = 34.5–37.5 °C, Invitrogen) in 1.5 mL water was added before lyophilization.

Hydrogels synthesis and characterization

To load polyplexes into HA hydrogels through CnE, lyophilized polyplexes were reconstituted with 100 µL HA-AC solution (in 0.3 M triethanolamine or TEOA, pH = 8.0) containing crosslinker, dithiothreitol (DTT) and gelled through incubation at 37 °C for 30 min. To load polyplexes through the regular encapsulation process, DNA and PEI were mixed in the HA-AC solution, vortexed for 15 s and incubated for 15 min at room temperature before adding crosslinker to form hydrogels at 37 °C for 30 min. To encapsulate polyplexes into fibrin hydrogels through CnE, 100µL fibrinogen solution in PBS (5 mg/mL) was mixed with lyophilized polyplexes and initiated to form gels at 37°C for 20 min with thrombin (2 U/mL). For the regular encapsulation, DNA and PEI were mixed and incubated in fibrin solution for 15 min at room temperature before adding thrombin to form hydrogels at 37 °C for 20 min. All hydrogels were swelled in PBS for 2-hrs. The swelled gels were stained with ethidium bromide (12 µM) for 2-hrs before imaging with a fluorescent microscope (Observer Z1, Zeiss) or a confocal microscope (Leica TCS SP MP) to visualize the distribution of polyplexes inside hydrogels. To test the DNA release from the hydrogel, a 30 µL hydrogel with polyplexes loaded through CnE was incubated in 200 µL PBS (pH 7.4) at 37 °C. Medium was collected daily and released DNA was quantified with HOECHST dye (H33258) [27].

Choriallantoic Membrane (CAM) assay

CAM assay was performed as previously described [28]. Briefly, fertilized chicken eggs were released into tissue culture dishes after 3-days of incubation in a 38°C ventilated, humid egg incubator and were incubated in a sterile humid incubator at 37°C for another 10 days. Hydrogels with polyplexes were placed on the CAM away from major veins and incubated for 3 additional days. Gross pictures were recorded (Stemi 2000-C, Zeiss) before the embryo was infused with 1 mL of FITC-dextran (0.5 mg/mL in PBS, Sigma). The CAM with the hydrogel was cut and fixed in 4% paraformaldehyde for 2-hrs before imaging with a fluorescent microscope (Observer Z1 Zeiss). Fixed CAM was immersed in 37°C X-gal solution (5 mM Potassium ferricyanide, 5 mM Potassium ferrocyanide, 1 mM MgCl₂, 1 mg/mL X-Gal (Gold Biotechnology) in PBS) for 48-hrs to visualize the β-Gal expression, which was recorded with a color camera attached to a dissecting microscope (Stemi 2000-C, Zeiss).

Results

HA and fibrin induced the aggregation of DNA/PEI polyplexes

Directly mixing DNA/PEI polyplexes with a hydrogel precursor solution before gelation (regular encapsulation) resulted in severe polyplex aggregations (Fig. 1). Due to the soft,

loose and charged structures, DNA/PEI polyplexes tend to aggregate at high concentrations or in salt solution during their incorporation into hydrogels. We tested if HA or fibrin induce polyplex aggregation by measuring the polyplex size using dynamic light scattering (DLS) in the absence or presence of HA and fibrin molecules. The average diameter of DNA/PEI polyplexes in water was about 50–100 nm when the DNA concentration was low (5 μ g DNA in 100 μ L H₂O, N/P = 15). Adding 1% HA shifted the average diameter of the polyplexes to \sim 1 μ m. Severe aggregations occurred in the presence of 1% HA when the DNA concentration was increased to 50 μ g in 100 μ L as shown by appearance of precipitate and the disappearance of the particles within the solution (Fig. 1A). Polyplexes containing 100 μ g DNA (N/P = 15) were observed to aggregate into one large clot inside the 100 μ L HA hydrogel (Fig. 1B). Fibrin proteins also induced polyplex aggregation. The average diameters of DNA/PEI polyplexes containing 5 or 50 μ g DNA (N/P = 15) in 100 μ L 5 mg/mL fibrin solution were about 4.1 and 5.5 μ m respectively (Fig. 1C). Large aggregates were found when 100 μ g DNA (N/P = 15) were incorporated into 100 μ L fibrin hydrogels (Fig. 1D).

Encapsulating DNA/PEI polyplexes into HA and fibrin hydrogels through CnE

Caged nanoparticle encapsulation (CnE) was used to introduce DNA/PEI polyplexes into HA and fibrin hydrogels. Dilute DNA/PEI polyplexes were prepared in the presence of sucrose and agarose and lyophilized. The lyophilized powder containing polyplexes was reconstituted with the hydrogel precursor solution before being initiated to form the hydrogels. Using CnE, up to 300 μ g DNA (N/P = 15) was encapsulated into 100 μ L HA hydrogel crosslinked through Michael addition. The polyplexes were stained with ethidium bromide and visualized through fluorescence microscopy (Fig. 2A–C and G–I) or confocal microscopy (Fig. 2D–F and J–L). In contrast to the regular encapsulation (Fig. 1), no polyplex aggregations were found inside the HA hydrogels through CnE. Without DNA, the hydrogel scaffold had limited auto-fluorescence (Fig. 2A, B). Polyplexes were distributed throughout the whole gel (Fig. 2B, C). At high magnification, polyplexes were observed to be suspended as unaggregated nanoparticles (Fig. 2E, F). The distribution of the polyplexes inside the hydrogels was not completely homogeneous and there were some micro-domains with dense concentration of polyplexes. However, both inside and outside these micro-domains, the polyplexes were unaggregated. Similar distributions were seen when polyplexes with 50 μ g DNA at N/P = 15 were encapsulated in 100 μ L 5 or 15 mg/mL fibrin hydrogels through CnE (Fig. 2G–L). Without DNA, the fibrin hydrogel scaffold had no fluorescence (Fig. 2G, L). Polyplexes were distributed throughout the whole gel (Fig. 2H, I). At high magnification, polyplexes were observed to be suspended as unaggregated nanoparticles (Fig. 2K, L). The fibrin concentrations had no significant influence on the polyplex distribution.

For DNA/PEI polyplexes encapsulated in HA hydrogels through CnE, less than 1% DNA in the hydrogel was released in PBS over 3 days (Fig. 3A). Polyplexes were retained in the 5 or 15 mg/mL fibrin hydrogel as well as shown that no DNA was released in PBS during the 3 days incubation (Fig. 3B).

HA hydrogels with DNA/PEI polyplexes loaded through CnE were able to deliver genes *in vivo*

The ability of HA hydrogels loaded with DNA/PEI polyplexes through CnE to mediate gene transfer *in vivo* was assessed using a chorionic chick embryo (CAM) assay. Hydrogels loaded with DNA encoding for β -galactosidase (p β gal) or VEGF (pVEGF) were placed on top of the CAM. p β gal was transferred from HA gels to cells and resulted in β -galactosidase gene expression determined from the extensive blue X-gal staining (Fig. 4B), which was not found when hydrogels containing pVEGF were used (Fig. 4A).

The delivery of DNA encoding for VEGF was able to induce blood vessel formation in the CAM assay. HA hydrogels with polyplexes containing pVEGF were placed on top of the CAM for 3 days. pVEGF was transferred to cells and produced a high concentration of VEGF at the gel area, which resulted in hyperbranched neovessels (Fig. 5, arrows). VEGFs diffusing out the gel created a decreasing VEGF gradient around the gel and led to radial neovessels around the gel (Fig. 5, arrowheads). Gross evaluation of the blood vessels around the implanted hydrogels showed that hydrogel with no DNA (Fig. 5A) and hydrogel loaded with DNA polyplexes encoding for β -galactosidase (Fig. 5B) did not result in enhanced blood vessel formation. However, hydrogel containing DNA encoding for VEGF resulted in enhanced blood vessel formation around the implant site (Fig. 5C, D, arrow heads). Increasing the dose of DNA did not increase the level of angiogenesis around the implant. Evaluation of the blood vessels underneath/inside the hydrogel scaffold after perfusion showed enhanced micro-vessel formation for all hydrogels that contained DNA/PEI polyplexes encoding for VEGF (Fig. 5G, H, arrows). Hydrogels with no DNA, or p β gal resulted in no micro-vessel formation (Fig. 5E, F).

Fibrin hydrogels with DNA/PEI polyplexes loaded through CnE were able to deliver genes *in vivo*

We tested the ability of DNA loaded fibrin hydrogels to deliver genes *in vivo* using CAM assay. Extensive X-gal staining was only observed for the 5 and 15 mg/mL hydrogels loaded with polyplexes containing p β gal indicating successful gene transfer occurred from the scaffold to the cells (Fig. 6B, C). We examined the density of the X-gal stained cell nucleus at high magnification (Fig. 6E, F, white arrows). Within the view, the number of stained nuclei was 0, 9 and 11 for the gel with no DNA, 5 and 15 mg/mL gels with DNA (Fig. 6D–F), respectively. However, we failed to see significant differences between the 5 and 15 mg/mL gels after examining a larger area (data not shown).

The delivery of DNA encoding for VEGF was able to induce angiogenesis in the CAM assay. Gross evaluation of the blood vessels around the implanted hydrogels showed that hydrogel with no DNA (Fig. 7A) did not result in enhanced blood vessel formation. However, all hydrogels containing DNA encoding VEGF resulted in enhanced blood vessel formation around the implant site (Fig. 7B, C, arrow heads). Evaluation of the blood vessels underneath/inside the hydrogel scaffold after perfusion showed enhanced micro-vessel formation for all hydrogels that contained polyplexes encoding for VEGF (Fig. 7E, F, arrows). Hydrogels with no DNA resulted in no micro-vessel formation (Fig. 7D).

Discussion

Local delivery of DNA through a hydrogel scaffold can extend the application of gene therapy to tissue regeneration and cancer therapy [6]. However, the delivery of DNA/cationic polymer nanoparticles (polyplexes) using hydrogels has not been successful partially due to the aggregation and inactivation of polyplexes inside hydrogel scaffolds [28]. To overcome this challenge, we developed a strategy to introduce unaggregated and highly active DNA/PEI polyplexes to PEG hydrogel scaffolds [28]. Specifically, we have found that sucrose was able to retain the activity of polyplexes during lyophilization, which agrees with previous findings [30–32]. We also found that agarose could prevent polyplex aggregation during the gelation. We hypothesized this was due to an increase in the viscosity of the gel precursor solution and a caging effect of the agarose polymers to the polyplexes [28]. MMP degradable PEG hydrogels with concentrated DNA/PEI polyplexes loaded through CnE were able to deliver genes *in vivo*. In this study, we used the CnE to load DNA/PEI polyplexes into HA and fibrin hydrogels and tested their abilities to mediate gene transfer *in vivo*. HA and fibrin are extensively used as scaffolds for tissue engineering, tissue regeneration and wound healing [33,34].

Due to the large numbers of carboxylic acid groups on the HA backbone, HA polymers exhibit a high density of negative charge that can interact with the DNA/PEI polyplexes through two potential mechanisms. HA can neutralize the positive charges at the polyplex surface and eliminate the repulsive force between the polyplexes, leading to severe aggregation. Alternatively, HA can interact with the PEI polymers and replace DNA from the DNA/PEI polyplexes similarly to what is found with heparin, causing decomplexation [35]. In dilute solution, HAs induced polyplex aggregation as showed by the fact that 1% HA increased the average diameter of the polyplex from 50~100 nm to 1.0 μm (Fig. 1A). In a solution with high concentration of polyplexes, adding 1% HA completely precipitated the polyplexes (Fig. 1B). Fibrin proteins induced polyplex aggregation as well (Fig. 1C, D). Through CnE, polyplex aggregations were prevented in both HA and fibrin hydrogels (Fig. 2). The aggregation of nanoparticles occurs through three basic steps [36]. Nanoparticles are under random motion in solution that leads to frequent collisions. Part of the collisions results in aggregations when the collided particles fail to separate. We hypothesize that agarose can slow down the particle motion and reduce particle/particle interactions through a combination of increasing the viscosity of the hydrogel precursor solution and caging the polyplexes with the long agarose polymers, which minimizes nanoparticle aggregation. Although no aggregation was observed (Fig. 2), micro-scale domains displaying higher concentration of polyplexes were found inside the hydrogel scaffolds. Inside the microdomains unaggregated polyplexes were found. We hypothesize that these microdomains are a result of incomplete dissolution of the lyophilized powder during the formation of hydrogels, which keep the polyplexes trapped inside the microspheres [28].

Polyplexes were not free to diffuse inside the hydrogels as shown by the fact that no DNA was released from the HA or fibrin hydrogels in PBS (Fig. 3). Thus hydrogel degradation was required for the gene transfer from hydrogels to cells, which allowed highly localized gene delivery *in vivo*. We speculate that DNA/PEI polyplexes could be released from the scaffold during the hydrogel degradation uptake by cells surrounding the hydrogel or they could be uptake by cells infiltrating the scaffold. From our results using a reporter plasmid encoding for β -galactosidase (Fig. 4 and 6), for HA hydrogels it appears that gene transfer only occurs in the area of the hydrogel, indicating that the polyplexes are transfecting either infiltrating or very near by cells and not diffusion far from the site of implantation. In fibrin hydrogels transfection is observed at regions further from the implantation site. However, most of the transfection was still observed in the area of the hydrogel. In contrast, for MMP degradable PEG hydrogels gene transfer occur mostly at sites adjacent to the implantation site [28], indicating that for HA and fibrin hydrogels the scaffold retains the polyplexes at the implantation site and that cells can more easily infiltrate these scaffolds. We speculate that the trafficking of DNA/PEI polyplexes once internalized by cells occurs through similar pathways as have been observed for cells cultured in tissue culture plates [37].

Polyplexes encapsulated inside the hydrogels through the CnE process were found highly active *in vivo* as shown by the β -galactosidase expression (Fig. 4, 6). To ensure that the expressed transgene was present at sufficient concentration to induce an angiogenic response in and around the hydrogel, plasmid DNA encoding for VEGF was entrapped within the hydrogel using the CnE. An angiogenic response was observed in all CAMs that contained a hydrogel with pVEGF/PEI polyplexes entrapped through CnE. The angiogenic response extended out in a radial orientation toward the CAM (Fig. 5 and 7, arrow heads), suggesting that the expressed VEGF diffused out of the hydrogel area and created a VEGF gradient with the highest concentration near the hydrogel. In contrast, the vessels observed at the hydrogel area are highly branched neovessels without preferred orientations (Fig. 5 and 7, arrows), suggesting that the VEGF concentration at the gel area was high and relatively constant. We previously demonstrated that the encapsulation of polyplexes through direct

encapsulation and in the absence of CnE led to extensively aggregated polyplexes that were not active *in vitro* or *in vivo* [28].

Conclusion

In summary, polyplexes are known to aggregate in HA or fibrin solutions. The CnE process prevents polyplex aggregation during their incorporation into HA or fibrin hydrogels. HA or fibrin hydrogels with concentrated polyplexes loaded through CnE were able to deliver genes in a CAM model.

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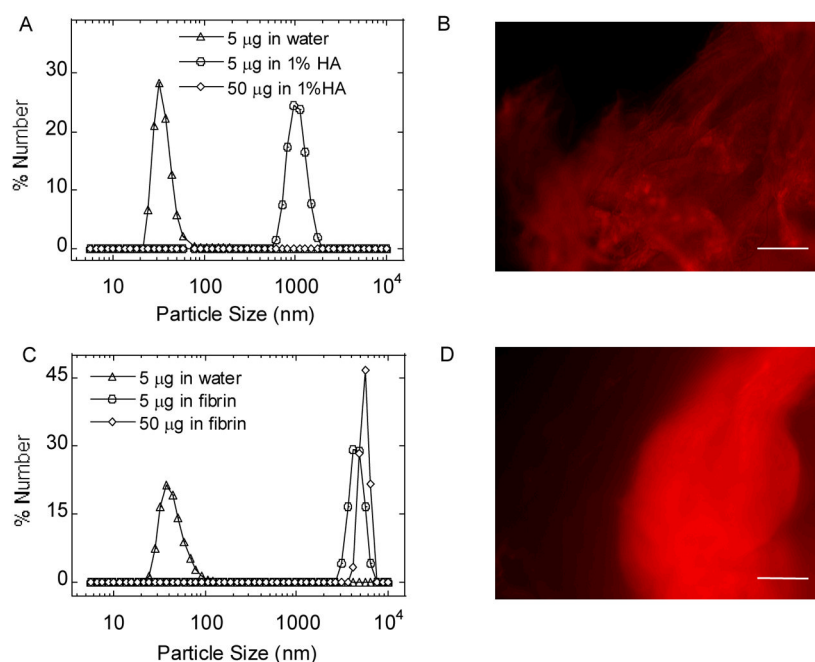


Figure 1.

HA or fibrin induced aggregation of polyplexes. (A, C) DNA/PEI polyplexes containing 5 or 50 µg DNA at N/P = 15 were prepared in 100 µL water or 1% HA or 5 mg/mL fibrinogen solution. The size distributions of the formed polyplexes were measured with DLS. (B, D) DNA/L-PEI polyplexes containing 100 µg DNA at N/P = 15 were encapsulated in 100 µL HA (B) or fibrin (D) hydrogels. The DNA was stained with ethidium bromide and visualized with a fluorescence microscope. Scale bar: 100 µm.

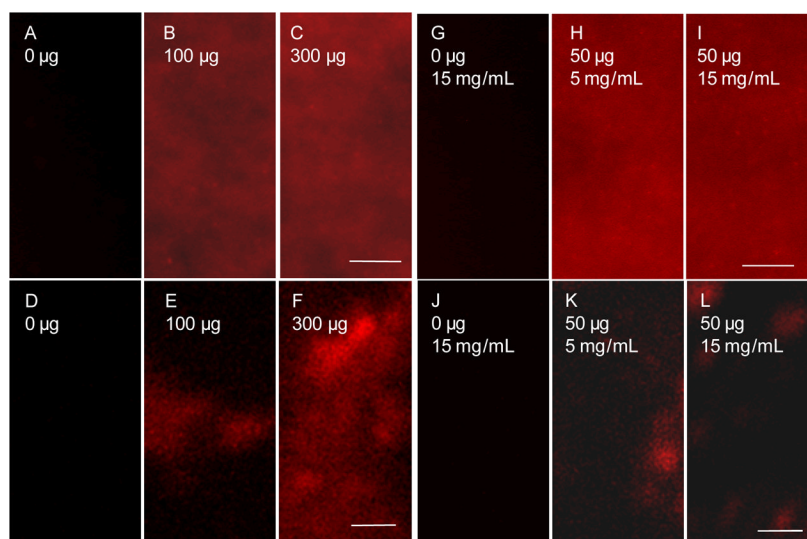


Figure 2.

Polyplex distribution in HA and fibrin hydrogels. DNA/PEI polyplexes containing 0 to 300 μg DNA at $\text{N/P} = 15$ were encapsulated in 100 μL HA (A–F) or fibrin (5 or 15 mg/mL) (G–L) hydrogels through CnE. The DNA was stained with ethidium bromide and visualized with a fluorescence (A–C, G–I) or confocal microscope (D–F, J–L). Scale bar: 50 μm for (A–C, G–I) and 1 μm for (D–F, J–L).

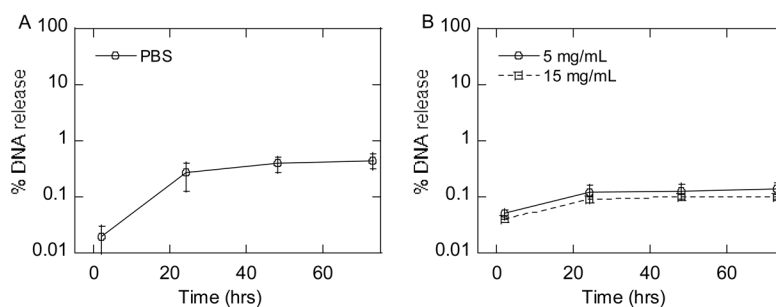


Figure 3.

DNA release from HA and fibrin hydrogels. (A) polyplexes containing 100 µg DNA at N/P = 15 were encapsulated into 100 µL HA hydrogel. The hydrogel was incubated in PBS (pH 7.4) at 37 °C. (B) polyplexes containing 50 µg DNA at N/P = 15 were encapsulated into 100 µL 5 or 15 mg/mL fibrin hydrogel. The hydrogels were incubated in PBS (pH 7.4) at 37 °C. The released DNA was quantified with HOECHST.

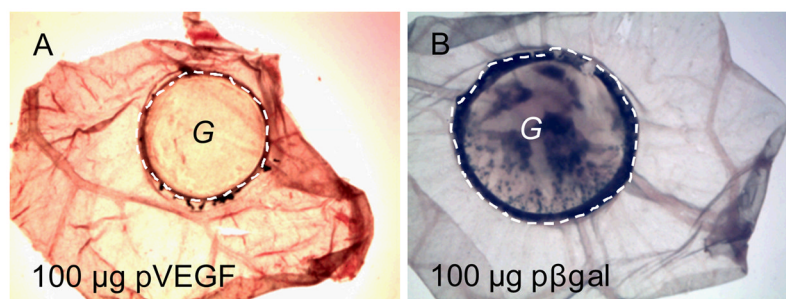


Figure 4.

DNA loaded HA hydrogels resulted in gene transfer in CAM model. A HA hydrogel with polyplexes was placed on top of CAM for 3 days. The gel with CAM was cut, fixed and stained with x-gal solution for 48 hrs. Positive β -galactosidase expression resulted in blue color. 100 μ g pVEGF or p β gal at N/P = 15 was loaded into 100 μ L gel. The dashed line highlights the edge of the hydrogel and “G” indicates the gel area

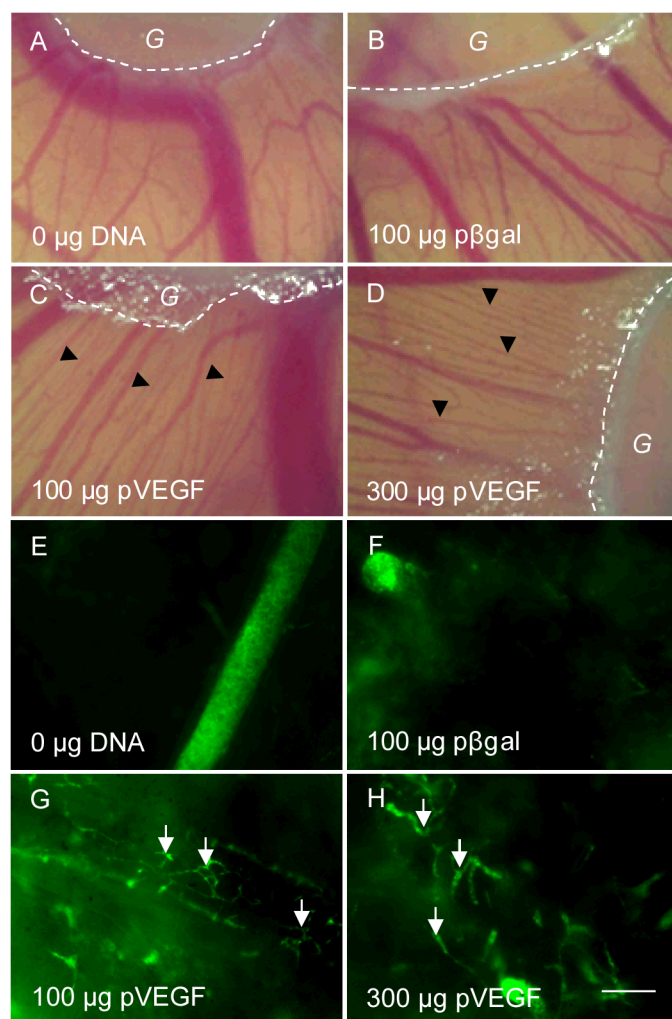


Figure 5.

pVEGF loaded HA hydrogels resulted in enhanced angiogenesis in a CAM model. A HA hydrogel with polyplexes containing pVEGF was placed on top of the CAM for 3 days. pVEGF was transferred to cells and produced a high concentration of VEGF at the gel area, which resulted in hyperbranched neovessels (arrow). VEGF diffusing out the gel created a decreasing VEGF gradient around the gel and led to radial neovessels around the gel (arrowheads). (A–D) Gross pictures on the gel edge were recorded before the CAM was infused with FITC-dextran for fluorescent imaging (E–H) at the gel area. Induced neovessels were found both around the gel (C–D, arrow heads) and at the gel area (G–H, arrows) with pVEGF, which were not found in the negative control (No DNA). Polyplexes at N/P = 15 were used. The dashed line highlights the edge of the hydrogel and “G” indicates the gel area. Scale bar: E–H: 200 µm

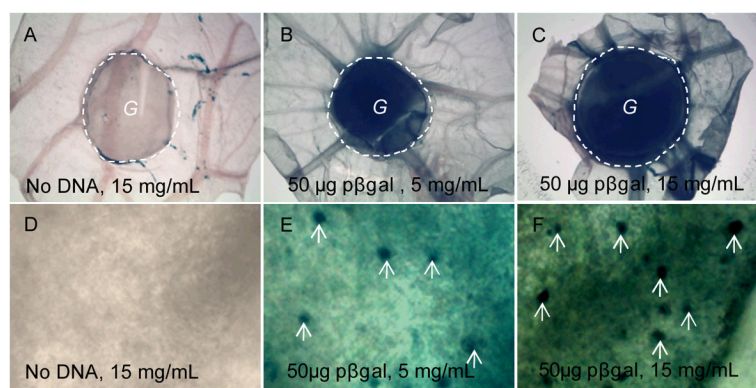


Figure 6.

DNA loaded fibrin hydrogels resulted in gene transfer in the CAM model. A fibrin hydrogel with polyplexes was placed on top of CAM for 3 days. The gel with CAM was cut, fixed and stained with x-gal solution for 48 hrs. Positive β -galactosidase expression resulted in blue color. (A–C) and (D–F) showed the low and high magnifications, respectively. 50 μ g p β gal at N/P = 15 was loaded to 100 μ L 5 or 15 mg/mL fibrin gels. The dashed line highlights the edge of the hydrogel and “G” indicates the gel area. The white arrows in (E and F) point out some of the stained nucleus.

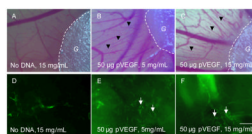


Figure 7.

pVEGF loaded fibrin hydrogels resulted in enhanced angiogenesis in a CAM model. A fibrin hydrogel with polyplexes containing pVEGF was placed on top of the CAM for 3 days. pVEGF was transferred to cells and produced a high concentration of VEGF at the gel area, which resulted in hyperbranched neovessels (arrow). VEGF diffusing out the gel created a decreasing VEGF gradient around the gel and led to radial neovessels around the gel (arrowheads). (A–C) Gross pictures on the gel edge were recorded before the CAM was infused with FITC-dextran for fluorescent imaging (D–F) at the gel area. Induced neovessels were found both around the gel (B–C, arrow heads) and at the gel area (E–F, arrows) with pVEGF, which were not found in the negative control (No DNA). 50 µg pVEGF at N/P = 15 were used for both the 5 and 15 mg/mL gels. The dashed line outlines the edge of the hydrogel and “G” indicates the gel area. Scale bar: D–F: 200µm.